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ENERGY SOURCES IN THE SEA URCHIN EMBRYO: A CRITIQUE OF CURRENT VIEWS

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INTRODUCTION

A student of the literature on the energy sources of the sea urchin embryo is apt to be overwhelmed by the amount of work devoted to this problem, and confused by the ambiguity of the results. These feelings are vividly experienced when reading the excellent and comprehensive reviews of YANAGISAWA (1975a, b).

Is energy supplied through glycolysis, or is it accomplished by the pentose phosphate pathway? Is energy supplied through a succession of different pathways? Are lipids oxidized by the mitochondria only during the later stages of development?

In the present paper we present the results of a number of experiments which might throw some light on the questions raised above. In fact, careful evaluation of the published data (YANAGISAWA, 1975a, b) shows that, unknown to the various authors, these questions have already been resolved. Thus, it is definitely excluded that carbohydrate to any appreciable extent is used for supply of energy through oxidation; there is not enough of it, and besides, we know that it is used also for synthetic purposes.

Although the available data are less than satisfactory, they rather suggest that lipids are the primary energy source during early, and probably during the whole, development. It will be understood that it is difficult to make *new* experiments in this field, and what is presented here is therefore mainly a repetition of earlier ones. However, they corroborate the conclusion drawn above, and show that previous authors may have misinterpreted their results.

MATERIALS AND METHODS

Eggs from *Paracentrotus lividus* were collected from excised ovaries, washed several times and fertilized. The eggs were rinsed free from sperm by several washes with sterile-filtered sea water and allowed to develop at room temperature (20-23º).

When the desired stage of development was reached, aliquots were collected and transferred into Warburg vessels containing 1 ml sterile sea water supplemented with labelled substrates. The pH was adjusted to that of normal sea water, and the concentration of the substrates was 0.09 mM, 0.5 µCi per ml.

The embryos were allowed to develop for another two hours at room temperature under gentle agitation in the Warburg vessels which contained 0.5 ml 1 N H_2SO_4 in the side arm and a piece of filter paper wetted with 50 µl 10% KOH in the central well. After the incubation, the reaction was stopped by tipping the sulfuric acid into the main compartment, whereupon the fixation of CO_2 was allowed to proceed for two hours. The filter papers were transferred to scintillation vials containing 1 ml H2O and extracted overnight. The radioactivity of the extracts was counted in the presence of 10 ml Scintix (CEA) as scintillation fluid in an Intertechnique liquid scintillation counter (model ABAC-SL 40). The results were corrected for background and quenching.

The amount of biological material in the aliquots was estimated by protein determinations.

Lipids were extracted and estimated in unfertilized eggs and embryos at prism stage (24 h of development) according to Bligh and Dyer (1959).

The following isotopes were used: ^{14}C-1-D-glucose (50.5 mCi per mmol), ^{14}C-6-D-glucose (51.2 mCi per mmol), ^{14}C-U-L-glutamic acid (250 mCi per mmol), ^{14}C-U-Na-acetate (98 mCi per mmol) and ^{14}C-2-Na-pyruvate (25 mCi per mmol). The isotopes were supplied by CEA, Paris.

RESULTS

Carbon dioxide production from various labelled metabolites was determined at seven stages during the first two days of development. Four to six determinations were made with each substrate at each stage. The results are shown in Figs. 1 and 2 and Table I. The $^{14}CO_2$ liberated from all substrates increases with development when expressed per mg protein of the biological material.

More CO_2 is produced from ^{14}C-1-glucose than from ^{14}C-6-glucose during the period studied, but the relative proportions vary. Thus, during segmentation the ratio C-1/C-6 is about 5, at the pluteus stage it is around 1.5.

The ratio between the activities of the glycolytic and the pentose phosphate (PP) pathways can be estimated from the relative yields of CO_2 from the two kinds of labelled glucose, the pentose phosphate shunt index being calculated from $1 - CO_2(C\text{-}6)/CO_2(C\text{-}1)$. The changes in this parameter are shown in Fig. 1, which indicates that glucose turnover to 80% passes through the PP pathway up to the stage of gastrulation. From then on, the PP shunt index decreases steadily, being around 25% in the pluteus.

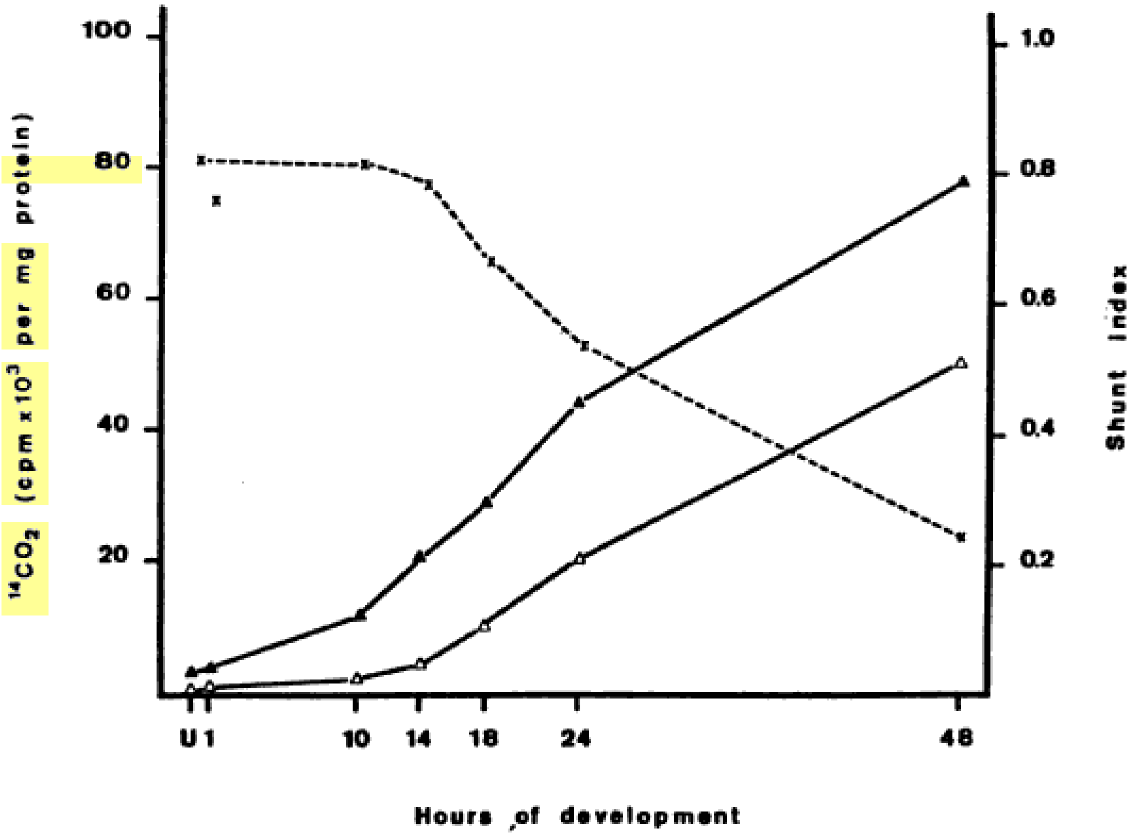


Fig. 1. — Radioactive CO_2 produced form glucose labelled in position 1 (▲) or 6 (Δ) and shunt index (x) during the development of *Paracentrotus lividus.*

Unfertilized eggs and embryos were incubated for 2 hours with 0.5 μCi labelled glucose, 0.09 mM, at room temperature. The experiments were performed as described in the text.

The PP shunt index indicates the participation of the pentose phosphate pathway in the turnover of glucose and is calculated as 1 - $^{14}CO_2(C\text{-}6)/^{14}CO_2(C\text{-}1)$. The developmental stages indicated refer to the beginning of the incubation.

Table I

CO_2-production from various substrates by the embryo of P. lividus, *expressed in nanomoles per mg protein and 2 hrs.*

	^{14}C-1-Glucose	^{14}C-6-Glucose	^{14}C-Acetate	^{14}C-Pyruvate	^{14}C-Glutamate/5	^{14}C-Glutamate/1
Unfertilized eggs	0.23	0.043	0.043	0.027	0.022	0.11
1 h. (1st division)	0.35	0.088	0.044	0.020	0.048	0.24
10 h. (blastula)	0.96	0.18	0.44	0.52	0.36	1.79
14 h. (gastrula)	1.71	0.39	0.50	0.66	0.48	2.42
18 h. (prism)	2.47	0.84	1.68	2.17	0.63	3.15
24 h. (early pluteus)	3.67	1.71	3.82	4.04	1.68	8.38
48 h. (pluteus)	6.48	4.58	4.37	4.67	1.98	9.91

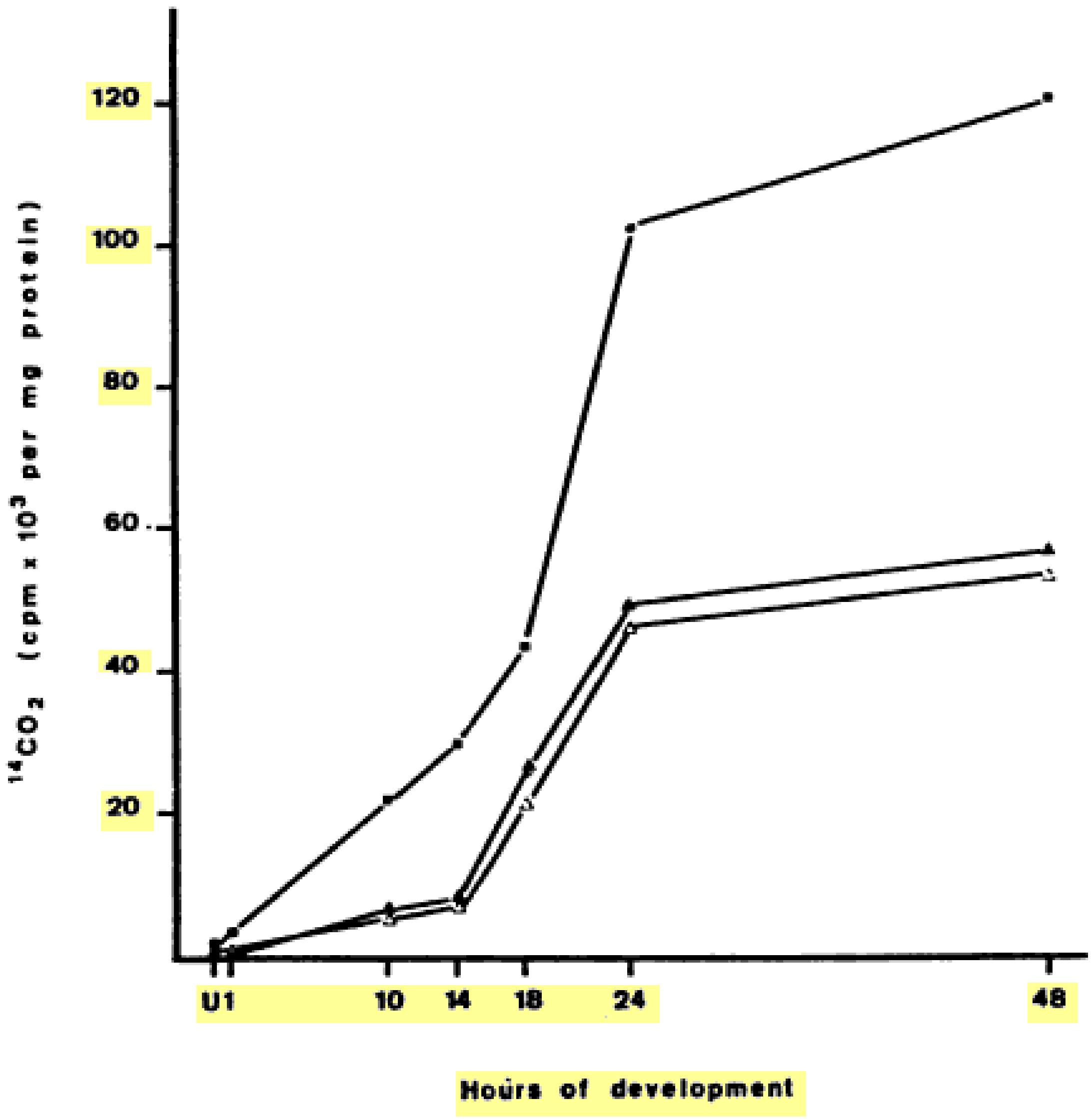


Fig. 2. — Radioactive CO_2 produced from labelled pyruvate (▲), acetate (Δ) and glutamate (■) during the development of *Paracentrotus lividus* embryos.

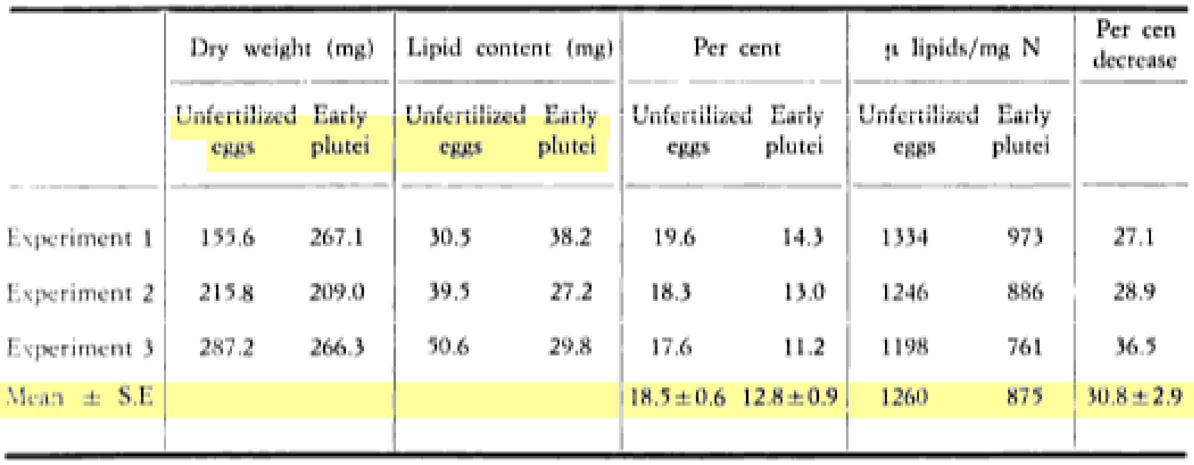
Experimental conditions as described under Fig. 1. Pyruvate was labelled in position 2, acetate and glutamate were uniformly labelled. The values for acetate have been divided by 2.

In Fig. 2 are summarized the results obtained with pyruvate, acetate and glutamate. The pyruvate used by us was labelled in position 2. This means that metabolic decarboxylation of pyruvate cannot be recorded unless the acetate thus formed, labelled in position 1, is further degraded, presumably in the tricarboxylic cycle.

It might be expected that this value coincides with that for acetate. As shown in Fig. 2 this was confirmed; except for the very early stages, the values for pyruvate and acetate coincide when correction is made for the fact that our acetate was uniformly labelled.

Table II

Lipid content of unfertilized eggs and of 24 hour embryos of P. lividus.



	Dry weight (mg)		Lipid content (mg)		Per cent		μ lipids/mg N		Per cen decrease
	Unfertilized eggs	Early plutei	Unfertilized eggs	Early plutei	Unfertilized eggs	Early plutei	Unfertilized eggs	Early plutei	
Experiment 1	155.6	267.1	30.5	38.2	19.6	14.3	1334	973	27.1
Experiment 2	215.8	209.0	39.5	27.2	18.3	13.0	1246	886	28.9
Experiment 3	287.2	266.3	50.6	29.8	17.6	11.2	1198	761	36.5
Mean ± S.E					18.5±0.6	12.8±0.9	1260	875	30.8±2.9

The relative production of CO_2 from glutamate decreases in the course of development. The implication of this will be discussed presently.

Table II shows the total lipid content of unfertilized eggs and of embryos after 24 hours of development. The results expressed per mg N are calculated on the basis of 100 mg dry weight = 14.7 mg N (ÖRSTRÖM and LINDBER, 1940). The lipid content decreases from 18.5% in unfertilized eggs to 12.8% in early plutei, which represents 31% of the total content.

DISCUSSION

The metabolism of the sea urchin embryo, in particular its energy metabolism, has been studied in many different ways, the most important being (1) chemical analyses of changes in the stores of potential energy sources, (2) indirect methods involving determinations of oxygen consumption and respiratory quotient and (3) investigations of the turnover of metabolites added to intact embryos or to homogenates.

The total carbohydrate content has been determined in five different species (*cf.* YANAGISAWA, 1975b); with one exception the values lie within the range of 10-20 mg glucose per g eggs (cfr. YANAGISAWA, 1975b). For *P. lividus* the value does not exceed 15; with 1 g eggs ~ 33 mg N, the carbohydrate content is around 450 g per mg N.

Certain authors have observed drastic and unexplained losses in association with fertilization, but the records for *P. lividus* indicate only a slight decrease during the first 10-12 hours of development.

The total lipid content in most species, including *P. lividus*, is close to 2,000 µg per mg N (ISONO and ISONO, 1975). These values lie above those observed by us, 1,260 µg per mg N. This discrepancy can be explained partly by methodological differences, partly by the fact that ISONO and ISONO (1975) calculated their results on the basis of 100 mg dry weight = 10.7 mg N instead of 14.7 mg N. If calculated with the former factor, the average lipid content in our experiments would be 1,730 µg.

The recorded decrease during development lies between 10 and 20 per cent, corresponding to 200-400 µg. In our experiments, the decrease in total lipids during the first 24 hours of development was on an average 385 µg per mg N.

WARBURG (1915) and LINDAHL (1939) have published curves for the changes in the respiratory rate during the embryonic development of *P. lividus*. The agreement between these results is quite satisfactory. Through an approximate integration of these curves for the first 24 hours one arrives at a total consumption of about 700 µl O_2 per mg N. The oxygen consumption during the remaining development up to the pluteus stage is 700-1,100 µl.

Knowing that 1 µg carbohydrate requires 1.83 µl O_2 for total combustion, the observed oxygen consumption corresponds to ~ 850 and 850–1,325 µg carbohydrate, respectively. Thus, the carbohydrates present suffice to support only half the energy consumption during the first 24 hours.

Since 1 µg lipid requires 2.02 µl O_2, the oxygen consumption during the first 24 hours corresponds to 350 µg lipid, and that for the remaining development to 350-550 µg. So according to these calculations somewhat less than one third of the lipid reserves may sustain the oxidative metabolism during the first 24 hours, in complete agreement with the result reported above.

These results thus support the conclusion submitted above, namely, that carbohydrates cannot be an important energy source. And this notion is further supported by the fact that glucose is utilized in the synthesis of nucleic acids (YANAGISAWA, 1975b) and glycosaminoglycans (NAKATSUJI and LØVTRUP, 1978; LØVTRUP-REIN and LOVTRUP, unpublished, taking place in the sea urchin embryo. The pentoses, hexosamines and hexuronic acids used for this purpose are made from glucose.

The amount of lipids present in the fertilized egg exceeds that needed for the total energy consumption. The amount consumed during the first 24 hours, assuming lipids to be the only source, is less than one third of the total amount.

How is it possible, with this background information, that most authors who have studied this question have contended that carbohydrate is an important energy source, accounting for more than half of the energy consumed (cfr. ISONO and YASUMASU, 1968)?

The answer to this question is that most studies have concerned metabolic pathways. A number of observations suggest that glycolysis does not occur to any appreciable extent in the early sea urchin embryo (cfr. YANAGISAWA, 1975b). Among these observations are: (1) hexosemonophosphates are hardly phosphorylated at all (KRAHL *et al.*, 1954), (2) hexosediphosphate does not stimulate the oxygen uptake (LINDBERG and ERNSTER, 1948), and (3) monoiodoacetate, an inhibitor of glycolysis, does not influence embryonic metabolism significantly (RUNNSTRÖM, 1935; LINDBERG and ERNSTER, 1948).

Seldom do biologists face more unambiguous data, and yet several authors have contested them (CLELAND and ROTSCHILD, 1952a; 1952b; YČAS, 1954). Their results definitely demonstrate that the glycolytic pathway enzymes are present in the embryo (cfr. also YANAGISAWA, 1975 b), but that does not imply, of course, that they are active.

The suspicion that glycolysis is of minor importance led to the search for alternatives. The results obtained by LINDBERG and ERNSTER (1948) suggest that the PP pathway may be involved. This point has been tested by studying the CO_2-production from glucose labelled in positions 1 or 6, an approach adopted by many authors (*e.g.* KRAHL, 1956; BÄCKSTRÖM *et al.*, 1960), and the results obtained are confirmed by ours (Fig. 1); the turnover of ^{14}C-1-G is much more rapid than that of ^{14}C-6-G during early development, but the difference is gradually reduced.

However, these results do not show that glucose is oxidized completely through the PP pathway, thus ensuring the energy supply in the embryo, for the method does not discriminate between the complete oxidation of glucose and its transformation into ribose-5-phosphate. To approach this question, glucose labelled in the 2 position has been used (KRAHL, 1956; BÄCKSTRÖM *et al.*, 1960). If glucose forms ribose-5-P, the label should remain in the sugar and no $^{14}CO_2$ be produced by this pathway, and the output should be comparable to that obtained with ^{14}C-6-G. If glucose is oxidized through the PP pathway, the output should be the same as for ^{14}C-1-G. In the quoted publications it was found that the CO_2-production from ^{14}C-2-G was comparable to, if slightly above, that obtained with ^{14}C-6-G.

It may thus be concluded that a substantial fraction of the glucose metabolized in the early sea urchin embryo is used to form ribose-5-P, while a minor fraction supplies energy via the glycolytic pathway and mitochondrial oxidation.

Our observations with acetate and pyruvate have some bearing on the question: carbohydrate or lipid? These results only show what the embryos *can* do, not what they actually do, but they show that the end product of lipid metabolism may be turned over at the same rate as that of carbohydrate metabolism, pyruvate. Thus, as far as the final oxidation through the mitochondria is concerned, the two energy sources are equivalent.

If the coincident values obtained for acetate and pyruvate represent the rate at which mitochondrial oxidation can occur, then it follows that the oxidation of glucose labelled in position 1 must take place extramitochondrially, partly at least. This inference concurs with the conclusion stated above (cfr. Table I).

Our results with glutamate indicate that it is engaged in a lively turnover, and this becomes understandable on the background of the results discussed so far. For glutamate is involved in the synthesis of purines and pyrimidines required for the synthesis of nucleic acids. And we therefore believe that the situation is the same for glutamate as for glucose: it is involved in synthetic reactions, being combusted at most to a very slight extent. The relative decrease in turnover of glutamate in the course of development, parallel to that of glucose through the PP pathway, suggests that the synthesis of nucleic acids gradually becomes of less importance.

With respect to the glutamate results we face two alternative interpretations. If we assume that glutamate is oxidized completely, *i.e.* that all carbon atoms form CO_2, then the number of glutamate molecules catabolized is represented by the column Glutamate/5 in Table I, the values of which show that this turnover rate is within the mitochondrial capacity.

However, we have rejected the notion that glutamate is used as a fuel, and in that case the number of carbon atoms giving rise to CO_2 must be smaller than 5. If it is 1, we arrive at the values in column Glutamate/1, suggesting a rate of turnover too high to pass through the mitochondrial electron carried chain.

Our final conclusion is thus the following: *The metabolism of the early development is principally directed towards synthetic activities (nucleic acids, glycosaminoglycans and, we presume, proteins) and it is sustained energetically through combustion of lipids. Furthermore, a substantial part of the oxidation occurring in the early embryo bypasses the mitochondria.*

SUMMARY

Current opinion claims that carbohydrates are the most important energy source in the development of the sea urchin, it being combusted initially through the pentose phosphate pathway. Available data refute this view, the amount of carbohydrates is far too small; lipids must be the main energy source. This conclusion has been verified in the present paper.

Previous and present results showing the involvement of the PP cycle rather demonstrate its participation in synthetic activities. Glutamate is metabolized at a relative high rate in the early embryo, suggesting that even this substance is engaged in synthetic activities.

Acknowledgements – The present experiments were performed at the Station Zoologique, Villefranche-Sur-Mer. We express our gratitude to the members of the staff, in particular Professor ROGER LALLIER, for hospitality and helpfulness.

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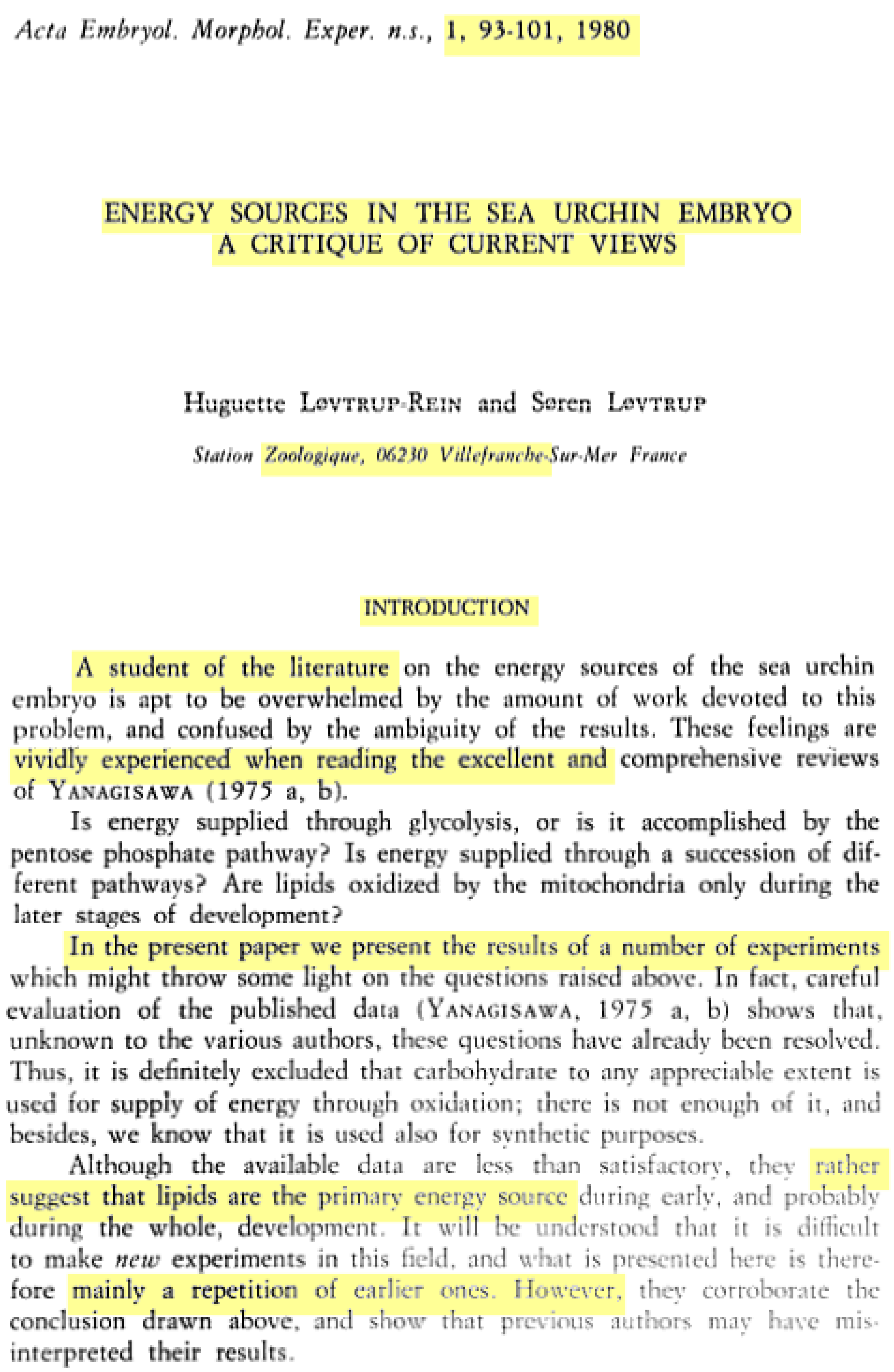

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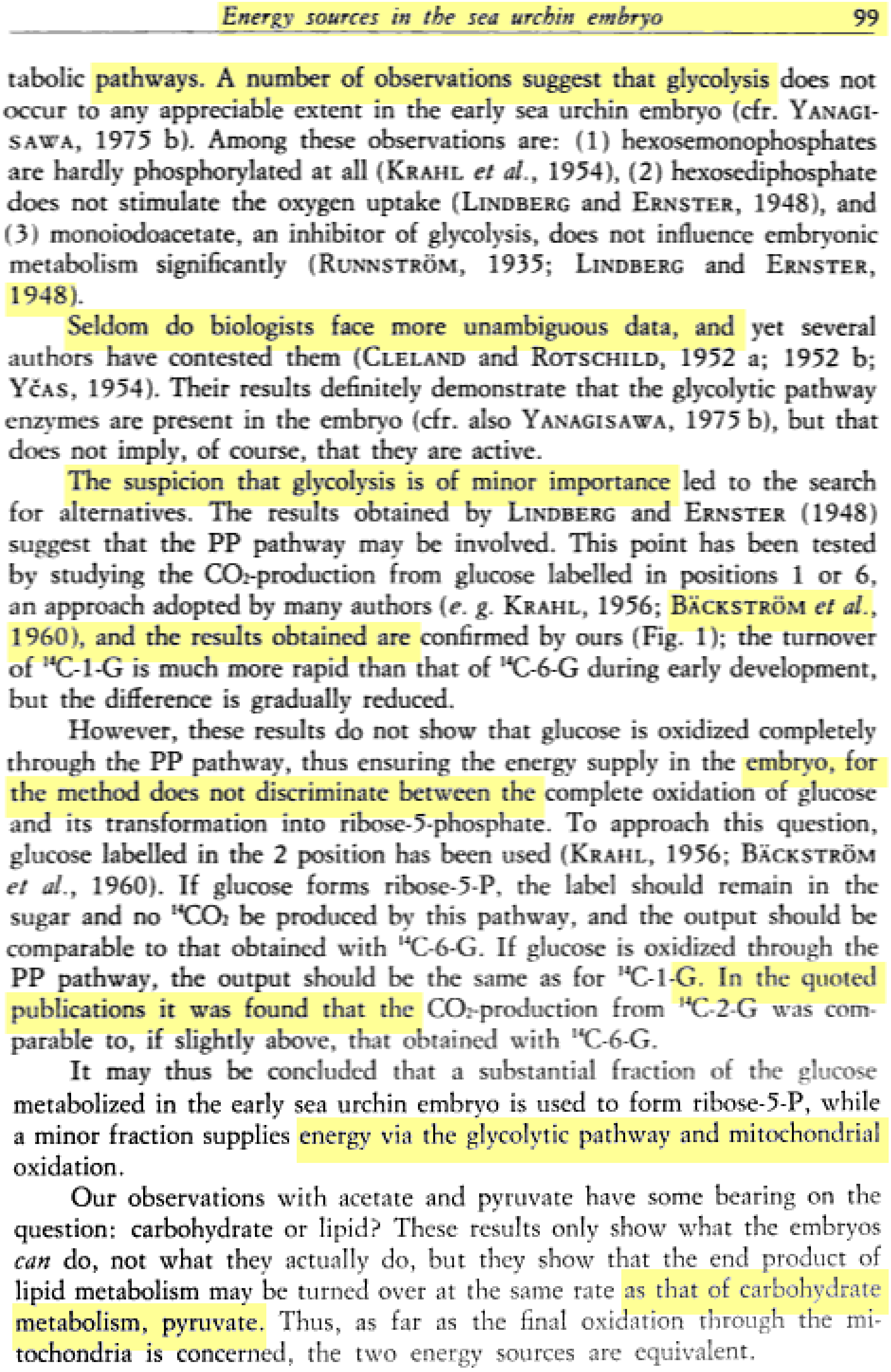

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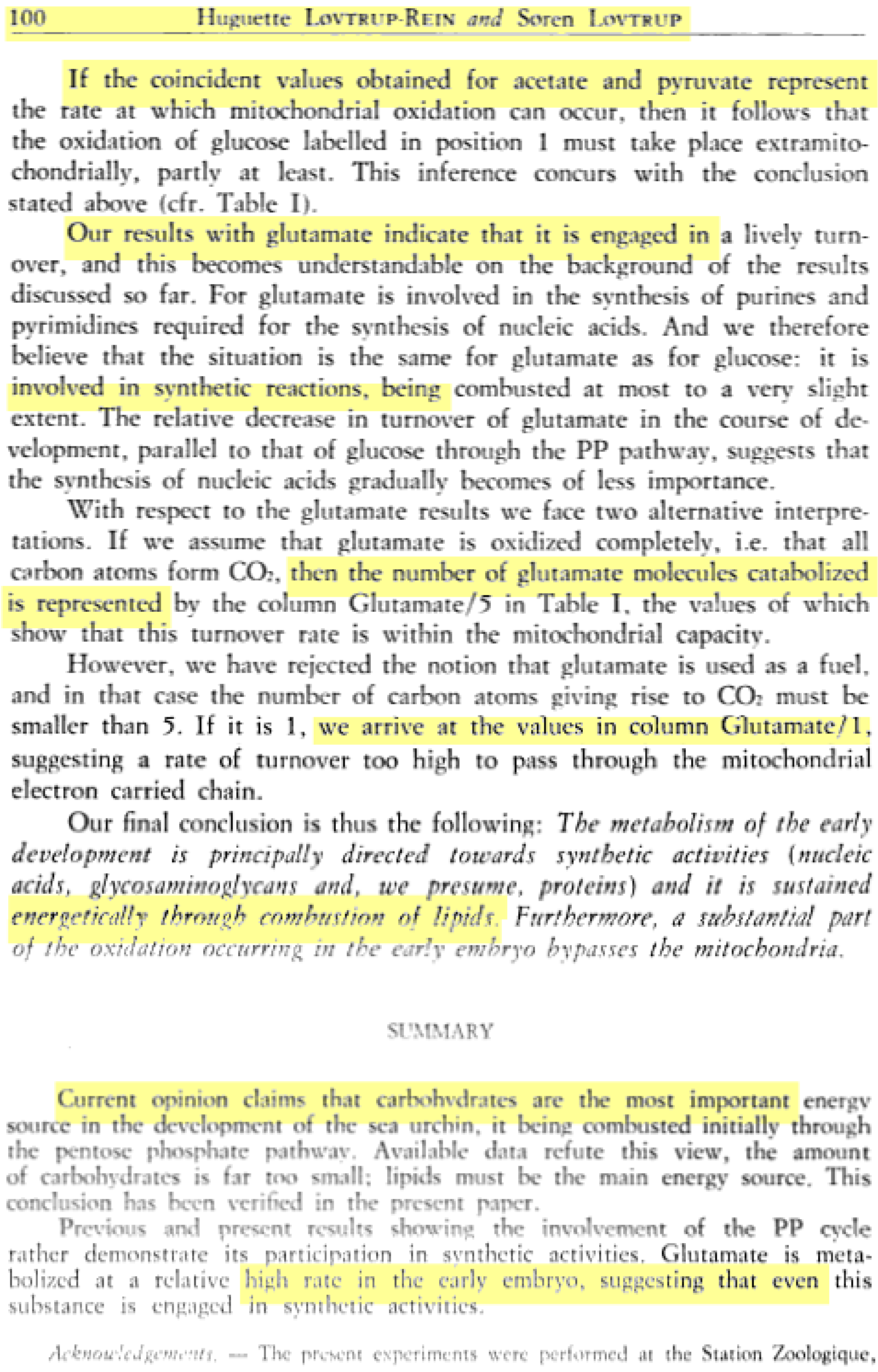

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SUMMARY

Current opinion claims that carbohydrates are the most important energy source in the development of the sea urchin, it being combusted initially through the pentose phosphate pathway. Available data refute this view, the amount of carbohydrates is far too small; lipids must be the main energy source. This conclusion has been verified in the present paper.

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